



# The role of soil layers in preventing ground water pollution with 17 $\beta$ -estradiol hormone (E<sub>2</sub>)

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## Abstract

**Background:** Estrogens include estoril (E<sub>3</sub>), estradiol and estrone (E<sub>1</sub>). These chemicals are produced in human and animal bodies as well as in synthetic chemicals (drugs). Estrogens can enter water sources in different ways. When these chemicals enter the human body through water and wastewater, they have the ability to mimic or disrupt the normal estrogen activities in humans and animals. Estrogens in wastewater are able to pass soil layers and contaminate groundwater. Therefore, in this study, the removal of the hormone 17 $\beta$ -estradiol (E<sub>2</sub>) as a representative of estrogens in three types of soils was studied. The selection was chosen in respect to the importance of entering the hormone into groundwater through the soil.

**Methods:** This study was an experimental study in which the removal of the hormone E<sub>2</sub> from different depths of three types of soils was experimented. The soils were consisted of two different textures, the silty sandy clay and the silty sand with gravel. The hormone E<sub>2</sub> was diluted and injected into the drilled holes. Soils were characterized in the soil mechanics laboratory. Hormone extraction from the soils was performed using a centrifuge and analyzed with the Elecsys device. The results were analyzed using the IBM SPSS version 22 software.

**Results:** The results showed that the removal rates of hormone E<sub>2</sub> in the three types of soils were higher than 99.5%, and the removal rate in the silty sand was more than the others. In all three soil samples, the removal rates in the first layer were high. The average injected hormone in the soil decreased from 3500 to 3112 ng/l. The results showed that the adhesion and plasticity of the soil had also affected the removal rates.

**Conclusion:** Results showed that the soil plays a significant role in the removal of E<sub>2</sub> hormone and this hormone was reduced or eliminated in the first layers of the soils. Thus, the risk of groundwater contamination is low.

**Keywords:** Estrogen, 17 $\beta$ -estradiol hormone, Silty sandy clay, Silty sand with gravel

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## Introduction

Hormones are a class of chemicals which are produced in human and animal bodies which regulate some main activities. These materials exist in the body at very low levels and any decrease or increase can have many adverse effects on the body's natural status, which may result in the development of some important diseases in human. In fact, estrogen is not the name of a particular hormone and it refers to a class of hormones which includes estoril (E<sub>3</sub>), estradiol (E<sub>2</sub>) and estrone (E<sub>1</sub>). Estrogens are mainly made in the ovaries and are known as gynaecic hormones. Men produce a small amount of this hormone in their testicles. Estrogens are responsible for the growth and extension of the female reproductive system, creation of gynaecic attributes, increase of ossification activities, distribution of body fats in gynaecic form, skin softness, elegance and

play a very important role during pregnancy and parturition. They enhance the beauty of women and draw attention to them (1).

Besides the human body, estrogens can be found in plants, synthetic chemicals, some pesticides like D.D.T, some medicinal combinations, detergents, plastics, ornamental materials, industrial materials and heavy metals like mercury, lead and cadmium (1,2). Estrogens entered the human body through water or wastewater have the capability to mimic or disturb the activity of natural existed estrogens in the body which have very dangerous consequences on human health such as testicle disorder syndrome, endometriosis, reduction of sperm number and very unfavorable complications on regeneration (infertility, cancers and metamorphosis) (2).

The appearance of gynandrous in fish and other aquatic



animals, the creation of unfavorable effects on the fertility of fish and mollusks are among the adverse effects of estrogens on aquatic animals (2,3). Besides the above unfavorable problems, another concern about estrogens is difficulty in specifying a threshold for their effects on more vulnerable populations (2).

Filtration methods such as activated sludge can be applied for removal of estrogens from wastewater resulted from refineries. Some filtration methods have removal efficiencies higher than 85% (2,4).

Studies have shown that treatment with chlorine and ozone are able to remove 80% to 95% and 95% to 99% of estrogen from water respectively (5).

Studies revealed that soil properties such as soil type and particle size have had effective roles on removal rates of impurities (6-9). The lateral flows of groundwater do not have any effects on the lateral relocation of any materials, but reinforce downward relocation (10). Soil depth is among parameters which affect material removal from soil (11). Parameters like volatility, contact surface with air, oxygen rate, soil porosity and moisture, primary density of impurity and atmospheric conditions, contact time and surface to depth ratio are among the factors affecting removal rates (12-15). The biologic removal of estrogens from soil is usually accomplished through the activity of bacterial or microbial consortiums and in some cases fungi and algae. Bacterial degradation is performed under aerobic and anaerobic conditions by iron, nitrate and sulfate bacteria (4). Biofilm layers also have the ability to remove  $E_2$  from wastewater (16). It has been shown that soil augmentation with *Bacillus* sp. PS11 can improve the removal efficiency by 20% (7). Organic materials (humic) present in the soil can hinder the mobility of estrogenic hormones and cause reduction of their presence and toxicity (17-19). The oxidation of soil organic material can also be effective on soil removal ability (20). The types of pollutants present in effluents or wastewaters are effective on estrogen relocation in soil (21). The soil senectitude phenomenon is another mechanism of removing estrogens from the soil. During senescing, some processes such as sorption to soil particles, being stockpiled in the spot and penetration to regions of soil which are unavailable (micropores) and stranding in soil organic material occur (14).

$E_2$  degradation can be similar to first-order reactions which lie in the range of  $0.006$ - $0.6\text{ h}^{-1}$  with relative factors and exist in testing materials with an average of  $0.026\text{ h}^{-1}$ . The calculated half-life of  $E_2$  has an average of  $47.6\text{ h}$  in soil samples and  $39.8\text{ h}$  in samples from soil colloids (17). In the environmental studies of estrogenic analyses of soil samples, there are urgent needs for accurate, rapid, comprehensive and sensitive distinction methods, since these pollutants are present in very low amounts and can interfere with a few analyses. The extraction of estrogens from soil samples is a difficult task because these combinations have a low solubility in water and are nearly hydrophobic combinations. Estrogens strongly stick to soil and their separation rarely occurs (14). Due to these reasons, few

assessment studies have investigated the level of estrogens in soil and their removing roles. All studies have been accomplished using high-performance liquid chromatography in their analysis. This study was performed with the aim of investigating the rate of  $17\beta$ -estradiol ( $E_2$ ) hormone removal from soil, using the human estradiol hormone recognition device, Elecsys.

## Methods

This research was an experimental study in an open-air location. Hormone  $E_2$  was used as a representative of estrogens. It was selected because of availability, low price and the existence of a measurement device for this hormone in medical recognition laboratories. Estradiol was diluted with diethyl ether solvent and reached a density of  $3500\text{ ng/L}$  (14). This value is considered equal to the utmost density of estradiol extracted from surface run-offs (2).  $10\text{ cc}$  of diluted hormone was poured into falcon tubes. Then three regions with different soils were selected and in each region, one part was chosen randomly and zoned. Thereafter, four holes were drilled in each region for more reliability and accuracy of results and statistical analysis. Four parts in each region were selected randomly, and some holes with diameter of  $10\text{ cm}$  and depth of  $60\text{ cm}$  were drilled (22). The type and characterization of each region's soil were determined in a soil mechanics laboratory. Soil saturation was performed by Lofran's method (23). After saturation and before hormone injection, a  $10\text{ g}$  sample of soil from drilled holes was collected for determining the presence or lack of estradiol in soil samples and diluted estradiol hormone was injected into the holes. Samples were taken after 48 hours from the bottom of each hole in  $5\text{ cm}$  distances from the bottom, i.e. depths of  $65, 70, 75, 80, 85$  and  $90\text{ cm}$  (depths  $0, 5, 10, 15, 25$ , and  $30\text{ cm}$  of bottom wells) up to  $10\text{ g}$  from each depth for hormone analysis (14,17). Collected samples were completely dried in environmental temperature and in the laboratory, each  $10\text{ g}$  soil sample was added to a pipe containing  $10\text{ mg}$  diethyl ether. Thereafter, samples were spun for 5 minutes and centrifuged at  $150\text{ rpm}$  for 20 minutes, then the organic layer was collected and passed through a filter paper (14). The hormone extracted from the soil was analyzed using a measurement device of estradiol hormone of Elecsys mark which is an extremely sensitive device for human blood estradiol measurement. To test for device sensitivity, a diluted sample of  $3500\text{ ng/L}$  was analyzed by the device which showed  $2499\text{ ng/L}$ , the accuracy of this measurement system is about  $\pm 5\text{ ng/L}$ . For more insurance from analysis results, some samples were randomly analyzed twice and the device showed the same results. All obtained results were analyzed by IBM SPSS version 22 software.

## Results

The results of soil mechanics experiments showed that experimented soils included a type of silty sandy clay (CL-ML1) with Soil liquidity limit (LL) = 24 and Soil plasticity limit (PI) = 4, a type of silty sand with the gravel (SM)

with LL= 24 and PL = 3 and another type of silty sandy clay (CL-ML2) with LL= 27 and PI= 6. Table 1 shows the results of sample analysis after statistical analysis.

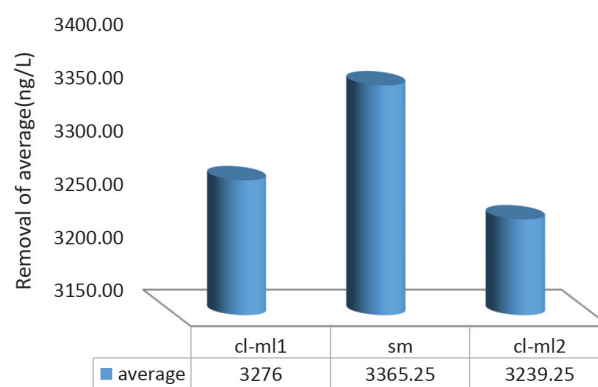
Table 1 shows the average and standard deviation (SD) values, along with minimum and maximum observed values and confidence interval of 95% for  $E_2$  removal in three types of experimented soils. As noted in the amounts of Table 1, silty sand with gravel (SM) with an average of 3365.25 (ng/L), by comparing to other types, had higher estradiol removal rates and the rates obtained in this type of soil showed a less deviation from the data average. Figures 1 and 2 show the results for intuitional perception. In order to compare the average  $E_2$  hormone absorption level in three studied soils with the assumption of its distribution normality in each group and performance of Levene test for investigating equality and homogeneity of variances, the unilateral variance analysis test was used which is explained in Table 2. Based on the results of Table 2, no significant difference was observed between average  $E_2$  hormone absorption level in three types of studied soils ( $F [2, 18] = 1.936, P > 0.05$ ).

Table 3 shows the average and SD values, along with minimum and maximum observed values and CI of 95% for  $E_2$  removal in various depths of the soil.

In order to compare the average  $E_2$  hormone absorption levels in various depths of the soils and performance of long test for investigating equality and homogeneity of variances, the unilateral variance analysis test was used and its result is explained in Table 4. Based on the results of Table 4, there is a significant difference between average  $E_2$  hormone absorption level in various depths of soils ( $F [6, 14] = 6.290, P < 0.05$ ).

The Dunnett test was performed in two by two comparisons of various depths with the final tested depth. The results of these tests are presented in Table 5 and groups which have significant difference with each other are marked with star mark. Based on the results obtained from Dunnett test, a significant statistical difference is observed between  $E_2$  hormone removal rate at the well bottom and at 5 cm with 30 cm depth ( $P < 0.05$ , but there was no significant difference in comparing other groups with the final studied depth ( $P > 0.05$ ).

Figure 3 has also been provided for comparing the effect



**Figure 1.** Estradiol removal average in three soil samples.

of soil depth on estradiol removal rate and indicates an increase of removal rate with depth increase in three types of experimented soils.

## Discussion

This research investigated the effect of depth, type and properties of soil on estrogen removal rate. The obtained results showed that the soil has a very effective role in the removal of  $E_2$  hormone and increase in penetration depth causes an increase in removal. The physical and chemical properties of soil such as particle size, plasticity limit and adhesion rate are among other effective parameters studied in this research. It was also shown that the removal average increases with increase in soil size; hence, soil samples with higher percentages of gravel and silt had higher penetration rates. Adhesion and plasticity limit in two types of soils with similar physical properties are among the factors affecting penetration rate. In this study, two types of silty sandy clay, the sample with higher adhesion and plasticity limit showed higher penetration rate. Ebrahimi et al (11) in their study investigated hydrocarbon pollution emission, they concluded with increase in penetration depth, the pollution rate rapidly decreases and this observation was also clearly observed in the present study.

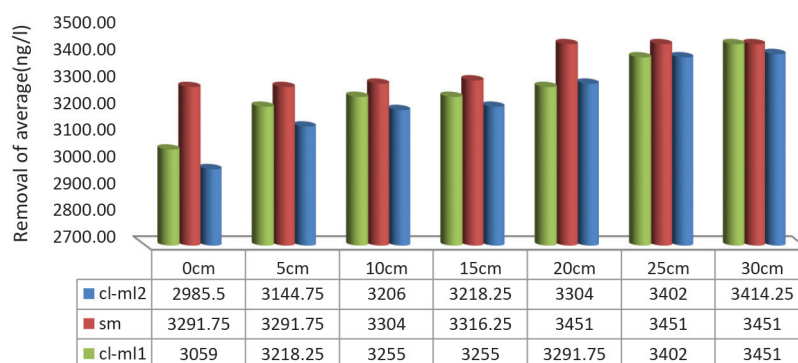
Gitipoor et al (24) in their study concluded that clay soils due to the special properties like high surface to volume ratio and high swell and penetration quality have always

**Table 1.** Values of descriptive indexes for estradiol removal in three types of soil (ng/L)

Type of soil	N	Mean	SD	SE	95% CI for mean		Minimum	Maximum
					Lower bound	Upper bound		
CL-ML1	7	3276.0000	127.83787	48.31817	3157.7697	3394.2303	3059.00	3451.00
SM	7	3365.2500	80.63937	30.47882	3290.6710	3439.8290	3291.75	3451.00
CL-ML2	7	3239.2500	150.64915	56.94003	3099.9228	3378.5772	2985.50	3414.25
Total	21	3293.5000	128.84564	28.11642	3234.8502	3352.1498	2985.50	3451.00

**Table 2.** Variance analysis test result in comparing the average estradiol removal in three types of soil (ng/L)

Source change	Sum of Squares	df	Mean Square	F	P value
Between Groups	58781.625	2	29390.813	1.936	0.173
Within Groups	273242.375	18	15180.132		
Total	332024.000	20			



**Figure 2.** Comparison of Estradiol removal average in three soil samples.

**Table 3.** Levels of descriptive indexes for Estradiol removal in various depths of soil (ng/L)

Depth (cm)	N	Mean	SD	SE	95% CI for mean		Minimum	Maximum
					Lower bound	Upper bound		
0	3	3112.0833	159.87697	92.30501	2714.9269	3509.2397	2985.50	3291.75
5	3	3218.2500	73.50000	42.43524	3035.6659	3400.8341	3144.75	3291.75
10	3	3255.0000	49.00000	28.29016	3133.2773	3376.7227	3206.00	3304.00
15	3	3263.1667	49.50779	28.58333	3140.1825	3386.1508	3218.25	3316.25
20	3	3348.9167	88.61868	51.16402	3128.7757	3569.0577	3291.75	3451.00
25	3	3418.3333	28.29016	16.33333	3348.0567	3488.6100	3402.00	3451.00
30	3	3438.7500	21.21762	12.25000	3386.0425	3491.4575	3414.25	3451.00
Total	21	3293.5000	128.84564	28.11642	3234.8502	3352.1498	2985.50	3451.00

**Table 4.** The result of various analysis test in comparison of average estradiol removal in three types of soil (ng/L)

Source change	Sum of squares	df	Mean square	F	P value
Between Groups	242186.583	6	40364.431	6.290	0.002
Within Groups	89837.417	14	6416.958		
Total	332024.000	20			

**Table 5.** The results of Dunnett test for two by two comparisons of various depths with the final tested depth

(I) Depth (cm)	(J) Depth (cm)	Mean Difference (I-J)	SE	P value	95% CI	
					Lower bound	Upper bound
0	30	-326.67*	65.40	.001	-517.15	-136.17
5	30	-220.50*	65.40	.021	-410.99	-30.00
10	30	-183.75	65.40	.060	-374.24	6.74
15	30	-175.58	65.40	.076	-366.07	14.90
20	30	-89.83	65.40	.577	-280.32	100.65
25	30	-20.41	65.40	.999	-210.90	170.07

been under consideration by environmental researchers for organic pollutant penetration. Among clay soils, bentonite types have special significance due to particular links between cells. Also, in the present study the rates of hormone removal in silty sandy clay soils were high.

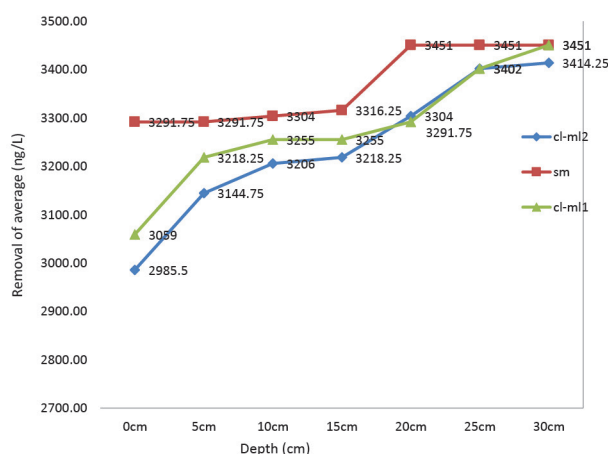
In the study of Mahmoodi et al (25), the rate of heavy metals removal from soils with smaller size particles was higher.

Cai et al (9) investigated the role of clay coarseness and softness on the rate of material removal. In this study, the role of soil cohesion on removal rate was investigated.

In study of Zadebafghi et al (15), they investigated parameters affecting the efficiency increase of cyanide natural decay and introduced parameters like volatility, contact

surface with materials, soil porosity and moisture, primary concentration and atmospheric conditions among factors affecting cyanide removal. They also concluded that for a distinctive soil volume polluted with cyanide, with increase of depth to surface ration, the amount of cyanide remaining in the soil decreases faster and higher primary density causes an increase of removal efficiency. In another study, Essandoh et al (6) reached the same conclusion. Fan et al (19) showed that  $E_2$  through abiotic chemical processes was converted to a polar unknown combination. Through physical and chemical processes, this hormone was oxidized to  $E_3$ . Other results showed that soil organic materials can inhibit the mobility of estrogenic hormones present in soil and as a result reduce their avail-





**Figure 3.** The effect of soil depth on Estradiol removal rate.

ability and toxicity. Based on the above research, we can conclude that estradiol used in this research was removed from the soil as a result of the chemical and physical reactions which occurred in the soil.

Cajthaml et al (4) studied the process of estrogens microbial removal in soil and showed that the process of removing these matters from soil, wastewater and septic is usually accomplished through the activity of bacteria and microbe consortiums and in some cases through fungi and seaweeds. Degradation is performed by bacteria under aerobic and anaerobic conditions and by iron, nitrate and sulfate bacteria and also through adsorption. In the performed study, the destruction of estradiol is likely to be achieved under aerobic and anaerobic conditions. Stanford et al (21) concluded that  $E_1$  and  $E_2$  present in septic effluent entering into the soil can be slaked with removal of surfactants containing phenyl polyethoxylate and total organic carbon. In this study, estradiol was diluted with aquapura and no other effective matter existed.

Chun et al (14) compared three methods of extraction of  $E_2$  in sand, bentonite and organic-rich silt loam and concluded that bentonite and silt loam had the highest efficiency of  $E_2$  extraction with diethyl ether method.

Chun et al (14) found that the extraction of estrogens from soil samples is a difficult task, since these combinations have little solubility and are nearly hydrophobic combinations. Estrogens strongly stick to soil and their separation rarely occurs.

Their final hypothesis was that diethyl ether due to having a polarity like estrogens, probably increases extraction efficiency. In the mentioned research, soil senectitude phenomenon was introduced as one of the probable reasons for  $E_2$  not being extractable from the soil. During this phenomenon, inter soil passive processes included sorption to soil particles, being stockpiled in the spot and penetration to soil regions that are not available (microprose) as well as stranding in soil organics (14).

Based on this research, diethyl ether was used for hormone extraction from soil and the obtained result showed that the amount of extracting hormone from experiments soils is very low. Prater et al (17) declared the average half-

life of  $E_2$  removal as 47.6 h in soil samples and 39.8 h in samples of soil colloids.

## Conclusion

Based on the results of the present study, soil plays an effective role in removing the  $E_2$  hormone and as a result, estrogens which have entered into the soil are reduced or removed in the primary layers of the soil. Therefore, the probability of polluting groundwater in regions where wastewater containing estrogens had passed through is very low.

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## Ethical issues

The authors certify that all data collected during the current study are presented in this manuscript and no data from the study has been or will be published separately.

## Competing interests

The authors have no competing interests.

## Author's contributions

MMT conceived and designed the study while AZ performed the literature search and wrote the manuscript. Both authors participated in data acquisitions analysis and interpretation. Both authors critically reviewed, refined and approved the manuscript.

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